Deposit structure and efficacy of pesticide application. 1: Interactions between deposit size, toxicant concentration and deposit number

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Abstract: Application of pesticides through a hydraulic nozzle produces deposits on a plant surface which have a spatial structure with elements of deposit size, number per area, and toxin per deposit. To investigate the relative contributions of these elements to the interaction of deposit structure and toxicant efficacy, we used a stochastic cellular automaton model of diamondback moth feeding on *Bacillus thuringiensis* (Bt)-treated cabbage – the Pesticide Dose Simulator (PDS) model. Data were analyzed using a specialized response surface approach called a mixture design. The advantage of this design was that it integrated the effects of deposit size, number per area and toxin per deposit on toxicant efficacy. Results from PDS simulations led to the following conclusions: (1) Deposit structure plays a major role in toxin efficacy. (2) Small deposits are not always the most efficacious. (3) Uniform coverage is not the best deposit structure if one is forced to limit application rates and field persistence. (4) Since uniform deposit structures allow an insect to live longer, uniform deposit structures should result in more insects acquiring sub-lethal doses. This may result in an interaction between 'uniform coverage' and the development of pesticide resistance in insect populations. (5) Percentage mortality and the level of crop protection are not necessarily correlated. Overall, these results help reconcile laboratory observations that small droplets are more efficacious with field observations that application of small droplets (eg from spinning disk sprayers) does not necessarily increase field efficacy.

Keywords: stochastic cellular automaton; modeling; mixture design; pesticide application; deposit structure

1 INTRODUCTION

The objective in the application of any crop protection agent is 'the placement on targets of just sufficient active ingredient to achieve a desired biological result with safety and economy'.[1] This statement clearly sets forth two goals. First, to apply sufficient active ingredient to achieve the biological result and, second, to apply as little active ingredient as possible consistent with the first goal. Research on the first objective clearly predominates in the literature, but research on identifying the minimum effective dose has not kept pace. This is due to the complexities inherent in identifying a minimum effective dose in the field (eg relating atomization to deposit formation and deposit quality to biological result), and the social and legal consequences of applying an ineffective dose. As a result we over-apply pesticides as 'insurance' of an effective application. However, over-application has come under attack due to concerns over pesticide impacts on non-target organisms, environmental contamination, and human health. Socially these have given rise to government mandates for pesticide reductions of 50% or more in Europe, and regulations like the Food Quality Protection Act (FQPA) in the United States of America. Thus, the social aspects of agriculture will ultimately force agriculture to use fewer pesticide applications, at lower rates, whilst maintaining productivity. To make this possible every aspect of the application process will require optimization.

We have focused on deposit structure as a key element in the dose transfer process because it is the interface between pesticide application and the target organism (Fig 1). While deposit structure can be measured over spatial scales from micrometers to kilometers, we have selected a range from 100 μm to 10 cm. This scale is appropriate because it is the scale at which individual insects, mites, and fungi encounter pesticides. At this scale, deposit structure is the distribution of pesticide on the target surface. It is measured as the arrangement of different numbers of deposits of different sizes on the target surface, along

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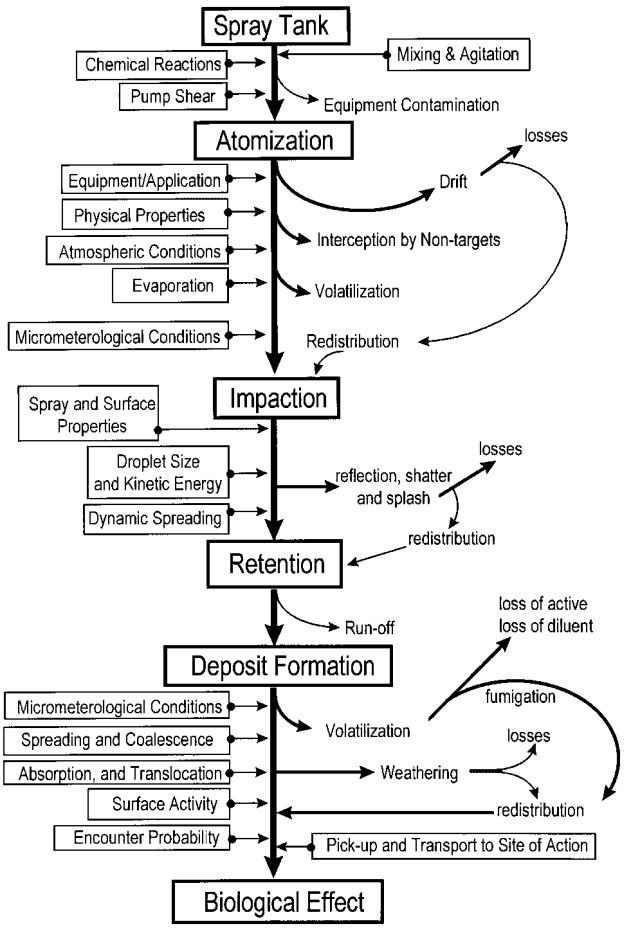

Figure 1. The dose transfer process showing the transfer of pesticide from the farmer to the target organism, and some important steps along the way.

with the dose per deposit. These features combine to produce a total dose per unit area.

Deposit structure has been measured and manipulated in many ways, both for experimental purposes and inadvertently through changes in application methodology. In field application, issues like coverage and application volume function through their effect on deposit structure. Coverage contains elements of deposit size and number which directly determine the proportion of the available area covered by the application. Changing application volume not only affects coverage, but also changes the concentration of AI per deposit given a constant application rate. However, the results of field and laboratory experiments provide conflicting evidence on the relationship between efficacy and deposit structure.

Coverage is the proportion of the target surface covered by the pesticide application. It appears to be a widely held belief that 'good' coverage requires 100% of the target surface covered by the application. This view is promoted by vague label recommendations such as apply in sufficient water for thorough coverage.[2] If all available surface is covered then the organism must either contact the pesticide or go somewhere else. Since the pest must encounter the pesticide for the latter to be effective, 100% coverage must be 'good.' However, for *Bacillus thuringiensis* Berliner (Bt) applied for control of *Ostrinia nubilalis* (Hübner) and *Hyphantria cunea* (Drury) increased coverage increased the LD_{50} for *O nubilalis*, and had a quadratic effect on *H cunea* (low LD_{80} at low and high coverage, with highest LD_{80} at the intermediate level of coverage).[3] While application methodologies which produce large numbers of small droplets (spinning disk, rotary cage, etc) often improve coverage on certain parts of the plant, a concomitant increase in efficacy is not necessarily observed.[4] Changes in application volume also change coverage. In general, larger volumes result in greater coverage per plant. For example, in applying Bt to oak against gypsy moth, increased volume resulted in increased coverage measured as $nl\,cm^{-2}$ foliage or $drops\,cm^{-2}$ foliage. However, increased coverage did not result in any increase in efficacy.[5,6]

Laboratory results suggest that with insecticides and acaricides, smaller droplet sizes are usually more efficacious: eg Bt–*Lymantria dispar* L;[7] bifenthrin–*Aphis gossypii* Glover;[8] permethrin–*Trialeurodes vaporariorum* (Westwood);[9] bifenthrin and dicofol–*Tetranychus urticae*.[10,11] However, in these studies, decreased deposit size was accompanied by an increase in deposit number. Therefore, increased number also results in increased efficacy. Other studies with Bt have also shown that smaller drops are a more effective use of Bt, in that fewer IU are required for a given level of mortality.[12] While smaller droplets are more effective, there is a lower size limit for Bt of about 40 to 80 μm. This is because droplets somewhere in this range no longer are able to deliver a high enough dose (even with pure formulation) and feeding inhibition allows the larva time to recover.[13,14]

Because different hardware for pesticide application changes the characteristics of the cloud of droplets produced (eg their size, velocity, retention) we looked for consistent differences in biological efficacy with different pesticide application methods. The hydraulic nozzle is often the most effective means of application. Hydraulic application was more effective than swing-fog machine or airblast in controlling mirids in cocoa.[15] Hydraulic, rotary cage (RC), and spinning disk (CDA) sprayers were tested against aphids in potato.[16] Aphids tend to favor the lower portions of plants, but despite increased deposition on the lower portions of the plant from the rotary cage atomizer, no improvement in efficacy was observed over the hydraulic nozzle. The CDA sprayer applied much more AI to the top portions of the plant, and less to the middle and lower parts. Thus, it is not entirely unexpected that the CDA sprayer did not perform better than the hydraulic nozzle.[16] In examining fungicide and herbicide application, CDA, RC, electrostatic, and electrohydrodynamic applicators were compared to hydraulic flat-fan nozzles. The hydraulic nozzles were the most biologically active method of application despite improved capture efficiency of targets when sprayed by the other methods.[17]

This review highlights two fundamental problems. First, better coverage does not always result in greater efficacy. Second, small deposits appear to be more effective, but application methodologies applying small droplets do not consistently produce better results. We suggest that the fundamental problem is in how we currently view deposit structure. What is needed is a new method for evaluating the effects of deposit structure. While many authors have identified significant effects of deposit size and number, or the associated effects of concentration (eg through changes in spray volume), none has synthesized these effects. In fact, it has repeatedly been stated that it is not possible to identify the effect of size independent of changes in droplet number (eg Bryant and Yendol[7]). We take a unique approach to the problem which provides answers to these questions for mobile (as opposed to sedentary) arthropods exposed to a gastrointestinal toxicant.

In this paper we examine the joint effect of size, number, and AI concentration on several measures of efficacy: percentage mortality, and quantity eaten. We use a Pesticide Dose Simulator (PDS) developed at LPCAT.[18–21] The PDS model is a stochastic cellular automaton simulating feeding and locomotory behavior of a defoliating insect on a leaf segment. The PDS model is a strategic model developed from data generated using diamondback moth (*Plutella xylostella* (L)) larvae feeding on Bt-treated cabbage. It was designed to develop underlying principles of pesticide dose-transfer from these initial data – as opposed to a tactical modeling approach which is to theorize, model the theory, and validate the model based on quantitative similarity between model results and 'real world' tests.

The PDS model was used to generate data for a response surface analysis using the method developed for analyzing industrial mixture data. Deposit size, number, and concentration were used to create different mixtures (or blends) of deposit structure all with the same total dose applied to the entire leaf segment. While this methodology is applicable to studying the effects of deposit structure for all pesticides, our results are most pertinent to mobile chewing insects that are being controlled with a toxin that has no sub-lethal effects and works only by ingestion. Because this methodology is unfamiliar to most biologists, we start by introducing it.

2. METHODOLOGY

2.1 Mixture methodology

A mixture is defined as a total made up of several components. Its critical features are the definition of a total, and that the components combine to produce the total. The independent variables (or components) are often expressed as a percentage of the total. For example, let $A = B1 + B2 + B3$, where A is the total (or 100%) while *B1*, *B2*, and *B3* are expressed as percentages ranging from 0 to 100%. If *B2* is 100% then *B1* and *B3* must be 0%, and so on. Thus, the components (or 'independent' variables) are not independent. This is in contrast to the standard factorial design. For example, consider the formulation of a pesticide where the formulation contains AI a surfactant, and a polymer, and one wishes to find the combination with maximal shelf life. One could run the experiment either as a factorial design or a mixture experiment. In the former, one would add 20, 50 and 100 ml of each factor to a flask and test the result – three factors each at three levels or nine total solutions. However, no matter in which order the components are added, the end result is a mixture of the three components in each liter of solution. Thus, one could also do the experiment using volume as a total which is expressed as a percentage (0 to 100) of each of the three components. An experiment where a total is expressed as the sum of several parts (and usually each part expressed as a percentage of the total) is termed a mixture design. The statistical analysis of mixture designs is a modified polynomial regression, and explained in detail by Cornel.[22]

2.2 Converting deposit structure to a mixture design

Our experimental design follows from the mathematical relationship between dose and application. Dose is related to deposit size, number of deposits per unit area, and concentration of AI per deposit as

$$D \propto R^3 NC$$

where D is the dose, R is the droplet radius, N is the number of drops per area, and C is the concentration. If D and C are held constant, then any change in R must result in some change in N. This violates the independence assumption required for use of factorial experimental designs. However, two changes must take place in order to use the mixture design approach: (1) the equation must be additive; (2) each component must be able to contribute from zero to 100% towards achieving the total. Performing a log transform of the above equation linearizes the relationship to

$$\log(D) \propto 3\log(R) + \log(N) + \log(C),$$

but there is still the problem of using percentages – to get 'log(C)' to vary from 0 to 100%. Begin by defining the minimum value of C (this is an arbitrary value, but see section 4 for additional detail), and dividing this minimum value by itself. Thus, at the minimum value $\log(C) = 0$, and one defines this as $0\,L^{\%}_{10}$.We use the $L^{\%}_{10}$ notation to try to avoid confusion between these 'percentages' and the more common use of the term percentage as used in mixture design methodology,[22] or when used as percent coverage or percent mortality. L stands for log transformed, 10 is the base, and % signifies that the variable is some fraction of a total quantity and can potentially contribute nothing to that total or can be the only element in that total. By repeating this process for the other variables, one

identifies the $0\,L_{10}^{\%}$ for them. Using the $0\,L_{10}^{\%}$ values, one can then calculate the $100\,L_{10}^{\%}$. Finally, one determines the application necessary for the $100\,L_{10}^{\%}$ treatment by taking the antilog and multiplying by the minimum value. This may give strange values for the $100\,L_{10}^{\%}$ treatment – such as applying a $3\,g\,ml^{-1}$, solution of a formulation with a density of $1.2\,g\,ml^{-1}$, or the need to apply 12 million droplets. These treatments force upper bounds onto the design space.

For example, assume the minimum number of droplets is one, the smallest size one can apply is 160 μm, and arbitrarily set the minimum concentration to $0.05\,g\,liter^{-1}$. The goal is to apply 2258 ng of a 19% AI formulation ($460\,ng\,cm^{-2}$) over a disk 2.5 cm in diameter. Note: values have been rounded to four decimal places, but calculations are done in extended precision. Round-off creates errors which are magnified as numbers are multiplied, divided, and transformed. The round-off for computation must accommodate the low values (0.05) of concentration as well as the large values for maximum droplet size or number.

(1) Minimum droplet size is then log (160/160) = 0, or by definition $0\,L_{10}^{\%}$.
(2) Maximum size is that required to contain all the AI in one droplet at the lowest concentration, which equals $(460/(1\star0.05\star2.5^{-2}\star(2/3)\star10^{-6}))^{1/3}$ or 4418.47876 μm (note: 10^{-6} converts centimeters (disk diameter) to micrometers (droplet diameter), area is expressed as $d^2\pi/4$, and the 1/3 power converts volume of liquid into micrometers in diameter).
(3) $100\,L_{10}^{\%}$ size is then defined as log(maximum value/minimum value) or log (4418.47876/160) = 1.4412.
(4) $50\,L_{10}^{\%}$ size is antilog $(1.4412\star0.50)\star160$, or about 841 μm.
(5) Repeat these steps for number and concentration to define the entire experimental region. Minimum number is 1, and minimum concentration is whatever dilution is experimentally reasonable. Reasonable is a balancing game among three factors: (1) unrealistically low dilutions; (2) quality of the estimated response, since the error increases rapidly at the edges of the response surface; (3) a very low concentration will result in large values for the other components. Unattainable values that force limits on the response surface have consequences in the experimental design.[22] From here on, $100\,L_{10}^{\%}$ of a component of deposit structure is the value from step 3.

There are four features of this system which require careful thought. First, the three variables 'size (ie deposit area or droplet diameter or volume)', 'number' and 'concentration' are interchangeable quantities. That is, one can exchange $2\,L_{10}^{\%}$ of size for $2\,L_{10}^{\%}$ of concentration. Second the application of a $0\,L_{10}^{\%}$ AI solution does not mean that one is applying a solution with no AI. Third, all variables, both dependent and independent are log transformed. Fourth, the shape of the untransformed surface is highly dependent on the minimum values chosen for size, number, and concentration (see Section 4 for details).

These steps are fundamental to using this methodology in a bioassay. In bioassays one uses droplet size (volume) to manipulate deposit size. In the PDS model one actually manipulates deposit size. As a result one can use the quadratic relationship between deposit diameter and deposit area in place of the cubic relationship between droplet diameter and droplet volume. This changes the shape of the response surface. In the diameter–volume relationship, the size axis is only one-third the length of the other two (in untransformed space), while in the diameter–area relationship it is only one-half.

2.3 Assumptions

We will make the following simplifying assumptions. (1) The deposit distribution is random over all target surfaces. (2) A uniform distribution of AI exists within each deposit. (3) One droplet produces one deposit which remains at the point of impact (no shatter, bounce, or run-off). (4) The pesticide does not redistribute following application (no translaminar activity, no translocation, no fumigant action, etc). This restriction eliminates 'zone of activity' effects.[23] While these may be important in many insecticides, they are not for insecticides like Bt which only act following ingestion.

The PDS model was set up with the following simplifying assumptions. (1) Insects are unable to detect the deposit. (2) Toxicant does not degrade. (3) Pesticide is active only following ingestion. (4) The only relevant behaviors are movement (behaviors not resulting in dose acquisition) and feeding (behaviors which may result in dose acquisition). (5) Movement and feeding are in a random direction, and for a random duration. (6) Deposits which cover the same portion of the target have additive AI in the area(s) of overlap. (7) One insect per simulation. (8) Behavior is not modified following sub-lethal ingestion of toxin (the PDS can model this effect, but that is another experiment with its own complexity). (9) Behavior is not modified by encountering leaf edges nor by encountering old feeding damage. (10) Dung does not accumulate – with no data on relevant behavior this has not been simulated. (11) Behavior is independent of photoperiod. All of these assumptions will be relaxed as our understanding of how deposit structure influences efficacy improves.

The restrictions we have imposed on the activity and mode of action of the pesticide are temporary limitations. This paper is about how to model the biological effects of pesticide application and provides an estimate of the importance of deposit quality in determining the biological effect of a toxicant. While the current scope is limited to the restrictions outlined above, we do not feel that the results are limited to

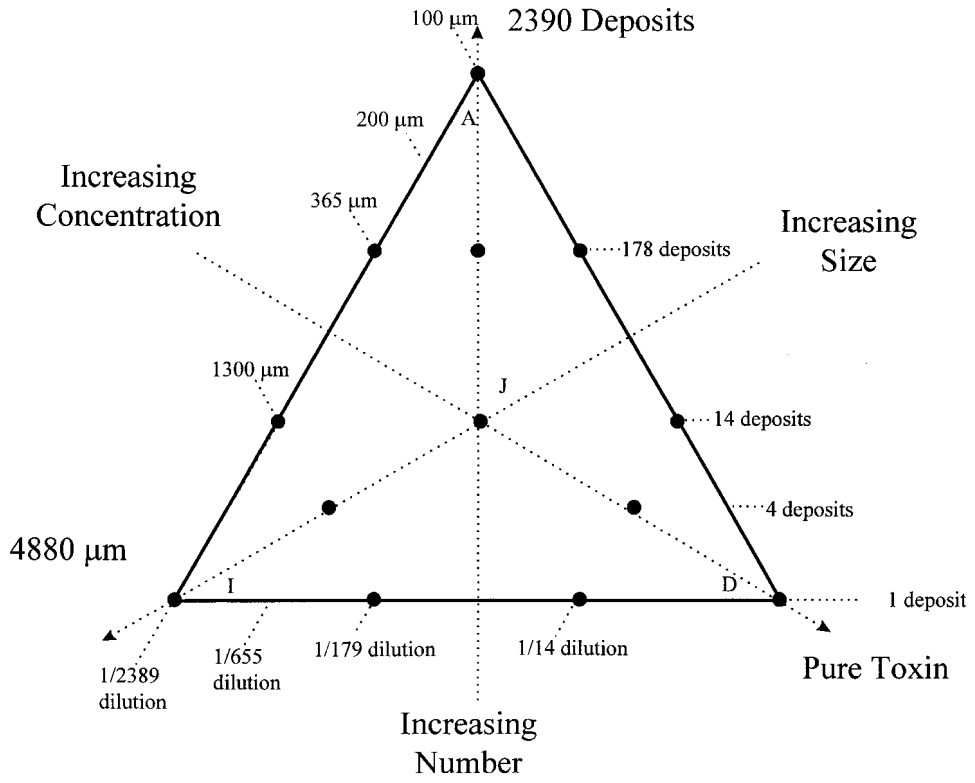

Figure 2. The treatment space for examining deposit structure efficacy using a mixture design.

products which behave like Bt. For example, a toxicant which has significant fumigant action may achieve a uniform toxin distribution in the boundary layer of the leaf. This should have all the advantages and disadvantages of a uniform deposit structure as predicted by our results.

2.4 Simulation experiments

Each experiment consists of 13 treatments distributed evenly over the entire experiment range (Fig 2, Table 1). For each treatment, the PDS model was run 1000 times. Percentage mortality was calculated using sets of 100 observations. Area eaten was averaged for the same set of observations. Thus the 'raw' data consist of 10 replicates from each of the 13 treatments. Data analysis and graphics were done using the industrial module of Statistica.[24] Since all treatments contained sufficient toxin to cause mortality, we assumed that no detected mortality indicated that the 'true' percentage mortality was below detectable levels. Consequently, 0.001 was added to percentage mortality prior to log transformation. To avoid taking the log(0) for area eaten, we added 1 to those values prior to transformation. The dependent variables were percentage mortality and the area eaten by the simulated larva. The model was run for 14 time intervals for each treatment (Table 1).

The total dose was five times the dose required to

Treatments	*Droplet size (µm)*	*Droplet number*	*AI concentration*[a]	Experiments	*Time (minutes)*
A	100	2390	1/2389	1	270
B	366	178	1/2389	2	540
C	100	178	1/179	3	1080
D	100	1	1	4	2160
E	365	1	1/14	5	2800
F	1306	14	1/2389	6	3240
G	182	4	1/14	7	3300
H	100	14	1/14	8	3740
I	4888	1	1/2389	9	4000
J (Centroid)	358	14	1/179	10	4320
K	1280	4	1/655	11	4590
L	191	178	1/655	12	5400
M	1339	1	1/179	13	6480
				14	8640

Table 1. Treatment and experiment list

[a] As a fraction of maximum concentration.

Table 2. PDS model behavioral states

Variable	Units	Range	Distribution
Feeding bout	Min	25–35	Uniform
Feeding interval	Min	20–30	Uniform
Feeding rate	Pixels min^{-1}	20–30	Uniform
Walking rate	mm min^{-1}	20–30	Uniform
Turning rate	Degrees per move	0–360	Uniform
Random number seed		123	Uniform
Droplet spatial distribution		mu = 3	Poisson

kill a larva. This is an arbitrary value which is expressed in this way because grams AI has little real meaning in a computer simulation, and it emphasizes the relationship between the AI applied to a plant surface and the AI required to kill the target organism. Table 2 lists the model parameter settings. Feeding bout is the duration of a feeding period in minutes. Feeding interval is the length of time in minutes between feeding bouts. Feeding rate is the number of pixels eaten per minute. Walking rate is the number of mm moved per minute. Turning rate is set so that there is no directional movement, the insect has an equal chance to go in any direction.

3 RESULTS

Mortality increased as larvae spent more time feeding on the treated surface (Fig 3). This figure shows the typical one-dimensional view of a time-response situation, but this graph also includes the range in mortality due to deposit structure. This range is maximal at about 4000 minutes where mortality varies from 1.8 to 88% depending on deposit structure. Mortality of 95% is first achieved in 4320 minutes which has a minimum mortality of 18.3%. The range in these estimates is about ±3% – thus another 1000 simulations might produce an estimate of the effect of deposit structure which would differ from the one shown here by no more than 3%. The range was determined by running additional simulations using a different random number seed (results not shown). Consequently the standard error in the plotted curves in Figs 3, 4, and 5 is no greater than the size of the symbols used in those graphs.

Time-response curves for selected deposit structures are shown for four of the 13 treatments (A, D, I, and J) (Fig 4). These are the vertices and center of the triangular response surface as shown in Fig 2. The response curve at $100\,L_{10}^{\%}$ number has a steep slope, but it takes a long time for this deposit structure to induce any mortality. During this time insects are acquiring sub-lethal doses. However, the biological response to this treatment is very uniform resulting in very low variability in individual response. The response curve at $100\,L_{10}^{\%}$ concentration is not a typical dose-response curve. The deposit structure of this treatment is a single small deposit. Thus the probability that the insect eats this dose equals the proportion of the plant eaten by the insect. Mortality is a linear function of the area consumed. The response curve at $100\,L_{10}^{\%}$ size is almost linear for the same reason it is at $100\,L_{10}^{\%}$ concentration. However, a slight deviation from linearity is imposed because the insect must eat a large quantity of the single deposit to acquire a lethal dose. Thus some insects will acquire a lethal dose by eating most of the deposit. Others must feed on the deposit, leave, and return several times to acquire a lethal dose. The response at the centroid is a fairly flat time-response curve which in some respects represents a kind of average response – size, number, and concentration are represented in equal portions. While this graph documents the effect of deposit structure on efficacy, it fails to show the interactions between the three components of deposit structure (size, number, concentration). For this, one needs to use a mixture design.

The effect of deposit structure on percent mortality

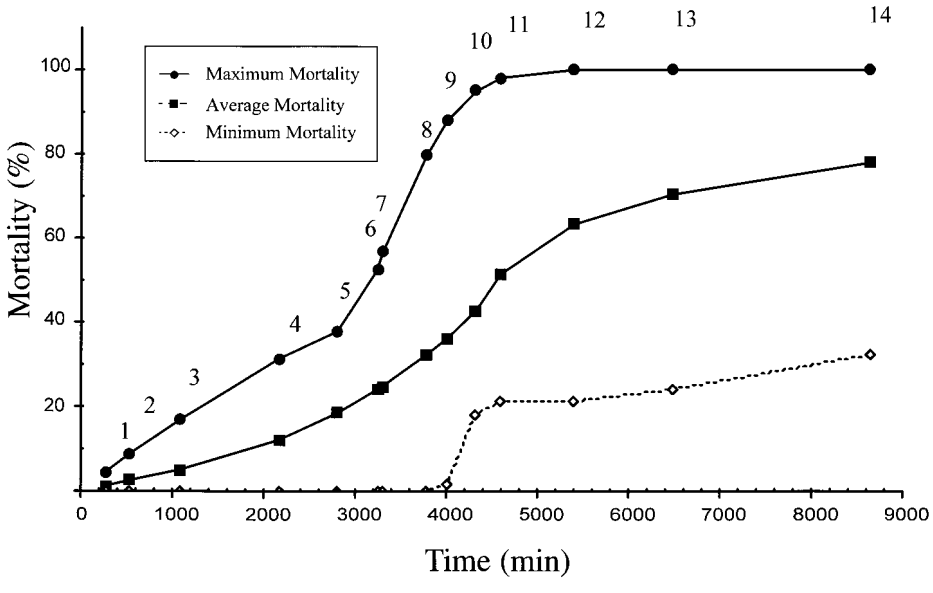

Figure 3. The average larval mortality time-response curve for an application of five times the lethal dose, plus the minimum and maximum response from our treatment list (Table 1).

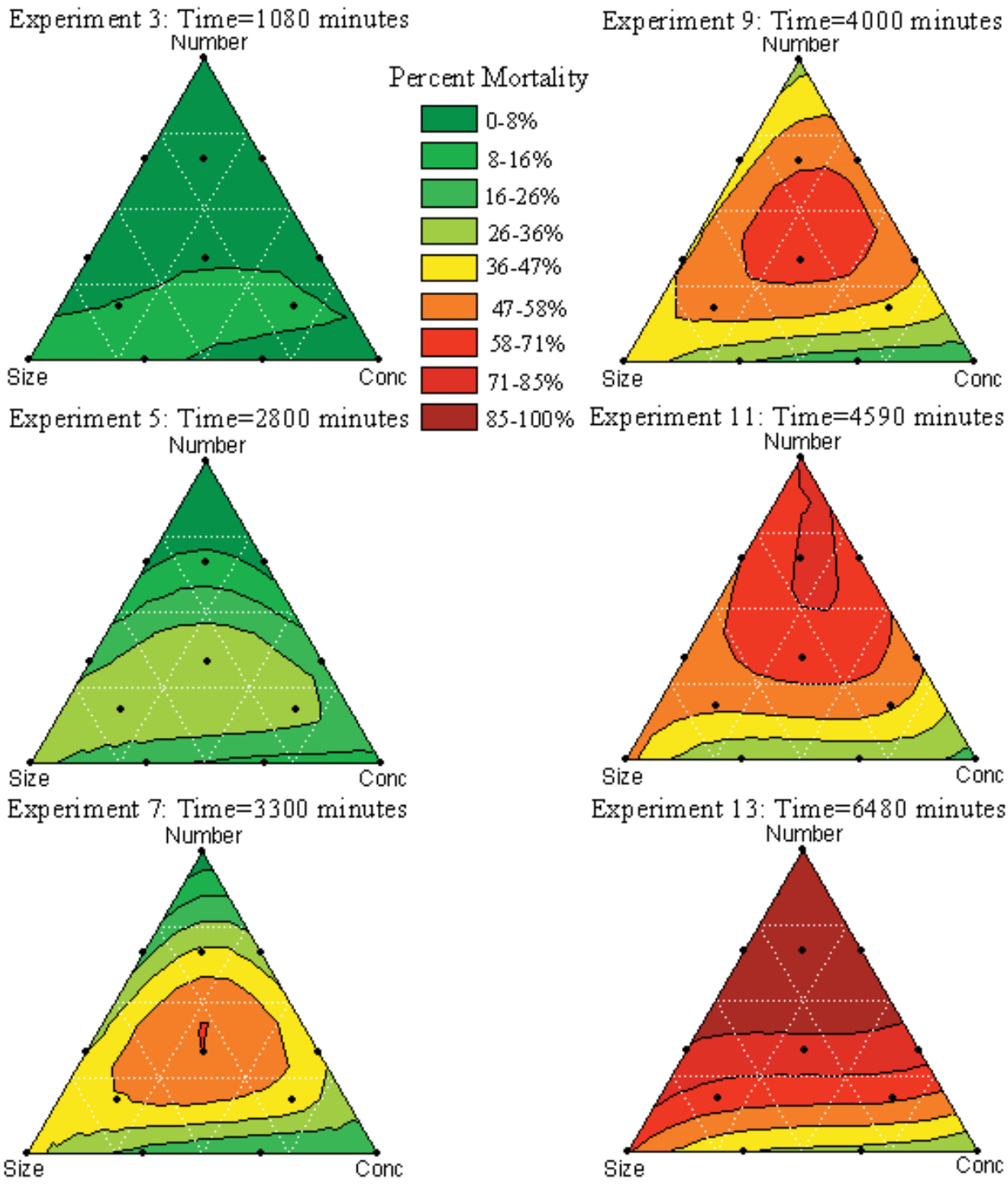

Plate 1. Effect of deposit structure on mortality at six time intervals.

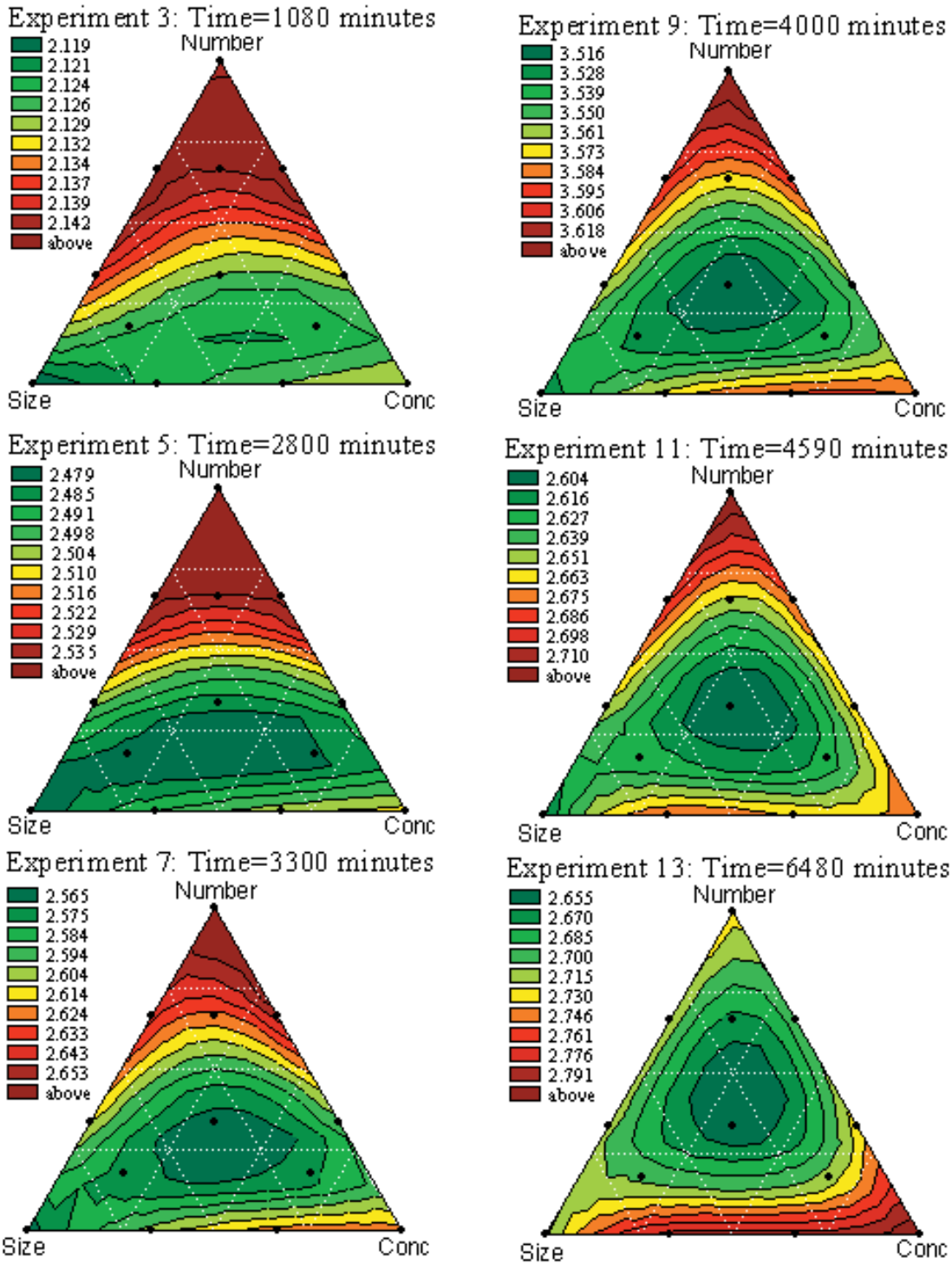

Plate 2. Effect of deposit structure on feeding damage at six time intervals.

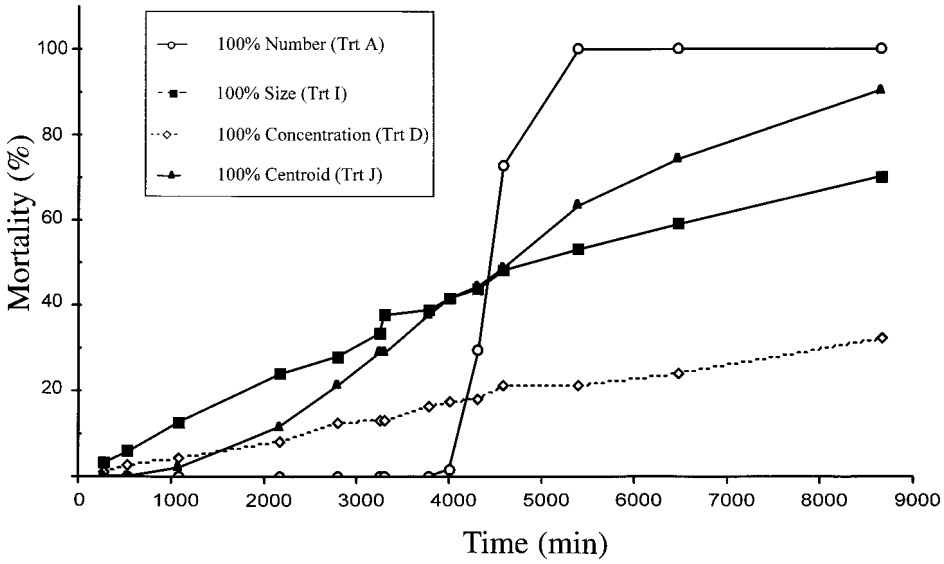

Figure 4. Time-response curves for selected deposit structures. Deposit structures A, I, and D are the vertices while J is the centroid (Fig 2).

is shown in Plate 1. The effect of deposit structure is minimal at very short and very long times. At short times there is little opportunity for response (almost flat response surface of experiment 3, Plate 1) while at long times eveything dies no matter what the deposit structure. At long time intervals, the pattern shown for experiment 13 (Plate 1) is shifted so that the 85–100% mortality interval covers an ever greater portion of the graph. While small deposits are more effective than large deposits, the smallest deposits are not the most efficacious. Thus one gets a bull's-eyes pattern at times 7 and 9. The most uniform coverage is produced by huge numbers of small deposits (the treatment at the very top of the graphs). This is not necessarily the optimal deposit structure because it takes longer for an individual insect to acquire a lethal dose than for any other structure. However, this deposit structure has the least variance in efficacy, and it has the steepest time-response curve of any deposit structure (Fig 4). This is because the dose is evenly spread over the entire surface, which results in all insects acquiring nearly the same dose per unit time.

Plate 2 shows the quantity of plant material eaten by the insect in the time interval of the simulation. The exact quantity of plant material eaten is not important, only where the maximum and minimum values are located within each graph, how these change over time, and how they relate to the mortality graphs (Plate 1). Experiment 9 (Plate 2), where mortality first exceeds 95% (Plate 1), shows that uniform deposit structures resulted in the highest levels of damage. Uniform deposit structures were still not the best even in experiment 13. However, one can see that the central area (with lowest damage) is moving towards the top of the graphs in experiments 9, 11, and 13. This trend continues and eventually uniform deposit structures do provide better protection. However, this does not occur within the simulated time span (six days). While there are some similarities between mortality and feeding damage (eg experiment 3), the relationship is tenuous at best (compare experiments 9 through 13). Lowest levels of damage occur more in the center of the graphs where high levels of mortality first appear.

The correlation between mortality and damage is shown in Fig 5. At very short and very long time intervals the correlation between damage and mortality is high. This is because, at short intervals, there is no mortality, and the level of damage is the same for all 13 treatments. At long time intervals, everything dies in most treatments, and the slow kill found with uniform deposit structures has been balanced by the long-term survivors in the more heterogeneous deposit structures. At the steepest part of the time-response curve (4000–4590 minutes) the correlation between mortality and damage is the weakest (smallest negative number). Since this is the target area (protection is high, rate is low) one must be clear on the objective: percentage mortality or crop protection.

4 DISCUSSION

In the introduction we suggested that better coverage does not always result in greater efficacy. Our results

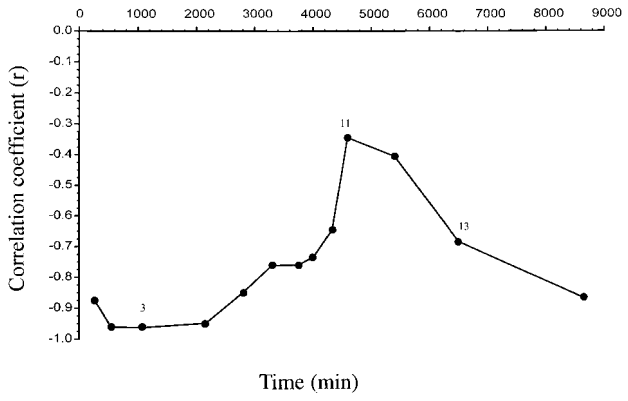

Figure 5. The correlation between mortality and feeding damage.

illustrate how this might happen. With a uniform deposit, insects can take longer to die, providing time for further growth, and consumption. In addition, AI in the deposit may decay and the plant may grow, both of which decrease the dose per unit area. Thus 'better' coverage provides poorer control. Of course this can be overcome by increasing the application rate (which shifts all curves in Figs 3 and 4 to the left), but that is not an acceptable solution in today's social and economic climate. The effects of coverage also include an issue of spatial scale. The spatial scale of a field is in kilometers, and at this scale uniform coverage is appropriate. The spatial scale of an insect may be from a few millimeters to a meter or so, and at this scale uniform coverage is less appropriate. This is because uniform coverage will increase sub-lethal exposure, increase survival times, and increase damage relative to other deposit structures. These results suggest that we need to apply a non-uniform deposit (on a leaf spatial scale) uniformly over the entire field (a hectare+ spatial scale).

We also stated in the introduction that small deposits appear to be more effective, but application methodologies applying small droplets do not consistently produce better results. From the field perspective this is a coverage issue. Previous laboratory results showing better efficacy with small droplets usually compared the effects of a small number of large droplets with a large number of smaller droplets (lines going from the bottom towards the right side in Fig 2 and plates 1 and 2 – (parallel to the left side as concentration is often held constant). Comparisons along these lines result in the conclusion that smaller droplets are more effective. Thus the two observations on the efficacy of small droplets in laboratory tests and field trials do not represent a conflict of results.

Not all papers showed that smaller droplets are beneficial. For example, bifenthrin in oil induced similar levels of mortality at all droplet sizes when applied to tobacco budworm larvae. However, larvae treated with small droplets (97 μm) lived longer than those with large droplets (337 μm).[25] This is consistent with our results where more uniform deposit structures resulted in longer survival times.

The goal of the application process should be to balance the probability of encountering a deposit with the probability of acquiring a fatal dose because deposit efficacy depends on the probability of contacting the toxin, the fraction transferred to the insect, and the probability of acquiring a fatal dose.[26] Encounter probabilities and mortality are functions of insect behavior and deposit structure; their balance may contribute to the development of resistance in insects. Clearly, uniform deposit structures provide greater opportunity for insects to acquire sub-lethal doses. This provides a methodology to re-evaluate the role of insect behavior in the development and management of pesticide resistance. Since insect behavior regulates the level of contact with the deposit, small heritable changes in insect behavior could be selected for. Such changes may then result in greater survival of parts of a population, and give these selected individuals additional time to develop physiological resistance. In such a scenario, resistant and susceptible populations of insects should show different behavioral characteristics.[19,27,28] However, deciding after the fact whether these behavioral changes promoted resistance development or are the result of physiological selection will be difficult.

This experimental approach is still in the development stage. In reconciling several previously irreconcilable results from the literature, we have partly validated our approach in developing the PDS model and the use of mixture design methods for exploring the efficacy of deposit structure. However, we must stress that this methodology is still under development: the primary goal of this paper is to present the foundation upon which we can develop more complex systems employing both simulations and bioassays. Because the treatments are expressed in logarithms, the selection of 0 $L_{10}^{\%}$ and 100 $L_{10}^{\%}$ values in the mixture experiment is arbitrary and interdependent. Any value of concentration can be selected as the 0 $L_{10}^{\%}$ value, and having selected a particular 0 $L_{10}^{\%}$ value changes the 100 $L_{10}^{\%}$ values for the other two variables. This may present special problems when examining dose-response or time-response curves with dose or time as process variables.[29]

Due to its importance, we would like to respond to one reviewer comment. There was some discussion about 'efficacy of deposit structure' versus 'influence of deposit structure on pesticide efficacy.' The question of which phrase to use is unimportant because they are equivalent, but the logic behind the phrasing is important. If one is to argue that deposit structure cannot have efficacy (possibly implicit in the second phrasing), then one must ensure a temporal component is always added to toxicant efficacy. Otherwise, one applies the same dose, but the biological response to this dose changes. That difference in efficacy must be attributed to something – hence deposit structure acquires efficacy. The individual graphs in Plate 1 promote the view of deposit structure having efficacy because they compress the temporal component into a single plane by asking 'how many are dead at a specific time?' rather than the temporal question of 'when did they die?' Only by viewing all the graphs as a single graph with a temporal axis can one visualize the temporal component in the average biological effect of the toxicant. Thus, the correct phrase might be 'influence of deposit structure on the rate of toxicant acquisition and the concomitant effects on pesticide efficacy.' However this phrase is long, and one could argue that time has efficacy (time = 0 has the same effect as dose = 0 on toxicant efficacy), and since deposit structure influences the rate of acquisition, it too can have efficacy.

Aspects of these results impinge upon all of application technology. If deposit structure can be manipulated to give even a fraction of the mortality

range observed here (20–90% for the same dose) it should be possible to manipulate deposit structure to improve efficacy and reduce application rates. This would reduce the human and environmental exposure to these chemicals, reducing human health risks and facilitating the integration of CPAs with more biologically based pest control programs. Furthermore, this approach to examining deposit structure applies to all pest control agents (insecticides, acaricides, fungicides, herbicides) where deposit quality influences efficacy (eg fungicides,[30] herbicides,[31–33]).

For ecological, social, and political reasons, those producing agricultural products will have to use less material while achieving the same or better results. This will require greatly improved targeting of the crop protection agent. We regard our approach of breaking down the application into four components (dose, size, number, concentration) as a first approximation towards understanding the fundamental principles of pesticide application and the dose transfer process. The practical consequence of the insight obtained here is that improved targeting can be achieved by optimizing deposit structure.

As these results show, the common objective of getting more with less is not an impossible ideal if we understand the system. We have little doubt that improved targeting of AI can result in both reduced rates and increased efficacy. However, we must be sure what we mean by efficacy. Our non-intuitive finding, that mortality and crop protection may not always be equivalent, points to a potential problem with current bioassay and field methodologies given that the overall goal is to reduce field application rates. If we mean more salable, less damaged commodity, then counting dead insects may misdirect research efforts aimed at improving crop protection. Our field efficacy and bioassay protocols should reflect the real goal of crop protection.

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